

Effect of Quinoid Mediators on the Decolorization of Azo Dyes by the Strain CD-2

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Abstract

In the present study, the effects of various quinone compounds on the decolorization rates of azo dyes by the *E. coli* strain CD-2 were investigated. The results showed that Lawsone was the most effective redox mediator. The optimum concentration for Lawsone is 0.1 mmol/L. The effects of physic-chemical parameters on the Methyl Orange degradation by the strain were determined. The results indicated that, in the quinone mediated decolorization system, strain CD-2 exhibited a good degradation ability in the range of pH from 4 to 9, temperature from 20°C to 50°C and salinity from 1% to 6%. With Lawsone as a redox mediator, a broad spectrum of azo dyes with different structures could be decolorized by the strain. All the results showed that the addition of a redox mediator can be valuable for treating dye-colored wastewaters.

Keywords

Azo Dyes, Anaerobic Decolorization, Anthraquinone Mediator

1. Introduction

Azo dyes, which characterized by one or more azo bonds, have been widely used in textile, food, cosmetics, paper printing and other industries [1] [2]. However, during production and application, about ten percent to fifteen percent untreated dyes discharge into environments with industrial effluents [3]. The characters of azo dye wastewater are high chroma, high concentration organics and high complicated components. Some azo dyes and their intermediate metabolites may cause cancer, malformation and mutation which seriously endanger the health of humans and animals [4]. Therefore, the different technologies have been developed for the effective treatment of azo dyes before emission.

Many techniques such as physical, chemical and biological processes are used to treat azo dyes. In particular, biological processes have received more interests due to their cost effective, lower sludge production and lower energy consumption [5]. It had been reported that many bacteria had the ability of decolorizing azo dyes under anaerobic conditions such as *Clostridium*, *Eubacterium*, *Escherichia* and *Bacillus* [6]-[9]. However, the bio-decolorization of azo dyes by bacteria often has problems such as slow decolorization rate and substrate limitation, which seriously hinder the industrial application of anaerobic biological decolorization of azo dyes [10].

Recently, many studies indicated that anthraquinone compounds (such as anthraquinone-2,6-disulfonate (AQDS), 2-anthraquinonesulfonate (AQS) and Lawsone) enhance the anaerobic decolorization of the azo dyes. Anthraquinone compounds can improve the decolorization efficiency of azo dyes by enhancing the electron transfer and avoid the sterichindrance of the dye molecules as redox mediators [11] [12]. In the azo dye decolorization system mediated by anthraquinone, bacteria are capable of utilizing different electron donors to promote reductive decolorization of azo dyes.

Therefore, we focus on the bacterial decolorization and degradation of azo dyes catalyzed by redox mediators and the further investigation to enhance the applicability of redox mediators on the bio-transformation of azo dyes.

2. Materials and Methods

2.1. Chemicals

The five azo dyes used in the experiments (Methyl Orange, Congo Red, Methyl Red, Eriochrome RedBand Ponceau S) were all procured from the Guangfu Fine Chemical Research Institute (Tianjin, China) and the four anthraquinone mediator (Lawsone, AQDS, Menadione and Bromamine Acid) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Methyl Orange was a sulfonatedazo dye and was selected as a model compound in this study. Some properties of the azo dyes and quinones used in this study were summarized in **Table 1**.

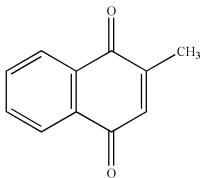
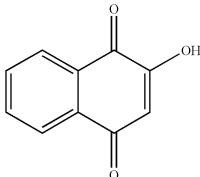
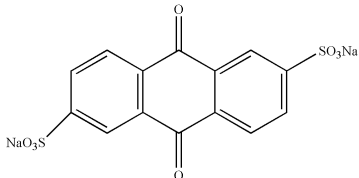
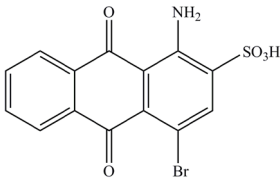
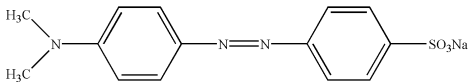
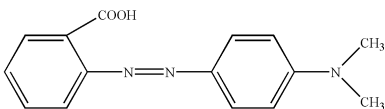
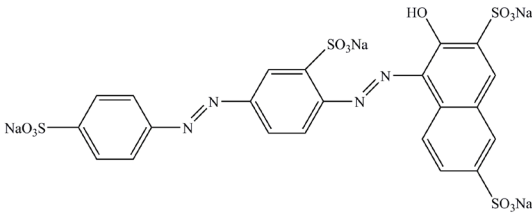
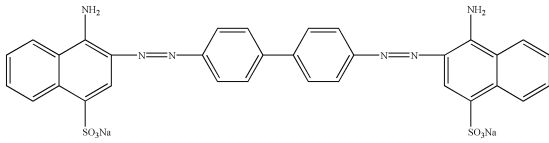
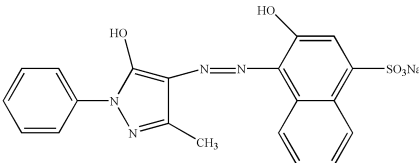
2.2. Bacterial Strain and Culture Medium

The *E. coli* strain CD-2 which had a high ability to decolorize azo dyes under anaerobic conditions was isolated by us from a wastewater treatment plant in Liaoning province. The degradation medium used in this study contained the following: 10 g·L⁻¹ glucose, 10 g·L⁻¹ NH₄Cl, 10 g·L⁻¹ KH₂PO₄, 10 g·L⁻¹ Na₂HPO₄ and 10 g·L⁻¹ MgSO₄.

2.3. Effect of Different Mediators on Anaerobic Decolorization of Azo Dyes

Strain CD-2 was grown aerobically at 37°C, 160 r/min until the OD₆₀₀ reached to 1.6 in the degradation medium. Bacterial cells were harvested by centrifugation (7000 r/min, 10 min). Cells were washed twice with 20 mmol/L phosphate buffer (pH 7.0) and suspended in 50 ml phosphate buffer. Then the cells were transferred to serum bottles. Oxygen was removed by repeated evacuation and flushing with nitrogen gas. 0.1

Table 1. Chemical structure of redox mediators and azo dyes used in this study.

Name	Chemical structure	λ_{max}	Solubility
Menadione		230	Water soluble
Lawsone		267	Water soluble
AQDS		450	Water soluble
Bromoamine Acid		492	Water soluble
Methyl Orange		464	Water soluble
Methyl Red		430	Alcohol soluble
Ponceau S		515	Water soluble
Gongo Red		500	Water soluble
Eriochrome Red B		474	Water soluble

mmol/L of Lawsone, AQDS, Bromamine Acid and Menadione were added to the anaerobic system, respectively. The final dye concentration was 0.1 mmol/L. The samples were taken at different time interval and analyzed for dye decolorization. All measurements were taken in triplicate.

2.4. Effect of Mediators Concentration on Anaerobic Decolorization of Azo Dyes

The strain CD-2 was inoculated under aerobic condition in the degradation medium until the OD₆₀₀ reached to 1.6. Cells washed twice with phosphate buffer and suspended in 50 ml serum bottles. Methyl orange was added to the degradation system to achieve a dye concentration of 0.1 mmol/L. Lawsone was added to the system as a redox mediator, and the concentration of the Lawsone was 0.05 mmol/L, 0.1 mmol/L, 0.15 mmol/L, 0.2 mmol/L and 0.25 mmol/L, respectively. Anaerobic bottles were incubated in 37°C. The samples were taken at different time intervals and analyzed for dye degradation. All measurements were taken in triplicate.

2.5. Effect of Physical and Chemical Factors on the Decolorization Rate of Azo Dyes

The strain CD-2 was inoculated under aerobic condition until the OD₆₀₀ reached to 1.6. Cells were washed twice with phosphate buffer and suspended in 50 ml anaerobic bottle. Methyl orange was added to the degradation system to achieve a dye concentration of 0.1 mmol/L. 0.1 mmol/L of Lawsone was added to the decolorization system as an anaerobic redox mediator. The degradation system was incubated under different physical and chemical conditions.

Effects of pH: The reaction mixtures were adjusted to pH values between 4 to 9 by using 1 mol/L HCl and NaOH.

Effects of temperature: The cultures were incubated at various temperatures (20°C - 50°C).

Effects of salinity: Various concentrations (1% - 6%) of NaCl were added to the media.

2.6. Effect of Mediators on Different Azo Dyes Decolorization

The strain CD-2 was inoculated under aerobic condition until the OD₆₀₀ reached to 1.6. Cells were washed twice with phosphate buffer and suspended in 50 ml anaerobic bottle. Methyl Red, Congo red, Eriochrome Red B, Ponceau S were added to the degradation system to achieve a dye concentration of 0.1 mmol/L. Lawsone was added to the reaction system as a mediator. The reaction system was placed in anaerobic culture of 37°C, and the decolorization efficiency of azo dyes was observed. All measurements were taken in triplicate.

3. Results and Discussion

3.1. Effect of Different Anthraquinone on the Anaerobic Decolorization of Methyl Orange by Strain CD-2

Strain CD-2 was tested for its ability to decolorize Methyl Orange under anaerobic

conditions with different anthraquinone. As shown in **Figure 1**, after 6 h of incubation, only 30% of the Methyl orange was decolorized without the addition of a redox mediator. However, in the presence of Bromamine Acid and AQDS, over 40% and 60% of the dye was decolorized in 6 h. In the presence of Menadione, over 90% of the dye was decolorized in 6 h (with a specific decolorization rate of 3.15 mg/g cell/h). Strain CD-2 decolorized 85% of the Methyl Orange in 3 h when the dye decolorizing mixture contained Lawsone (with a specific decolorization rate of 8.84 mg/g cell/h). The results demonstrated that the addition of quinoid redox mediators significantly increased the rate of decolorization of Methyl Orange under anaerobic conditions, and compared with other mediators, Lawsone was a more effective mediator.

The effects of different redox mediators varied significantly among different decolorization system. Previous studies demonstrated that the addition of AQDS to *E. coli* resulted in much higher azo dye decolorization rate under anaerobic conditions. Kudlich *et al.* reported that, for the strain *Sphingomonas xenophaga* BN6, the addition of AQDS on the dye reduction system allowed higher decolorization than other mediators [13]. In this study, Lawsone was the most effective mediator. In the presence of Lawsone, the decolorization rate of Methyl Orange increased about 8.5 times.

3.2. Effect of Concentrations of Anthraquinone Mediator on Decolorization of Methyl Orange by Strain CD-2

Previous studies demonstrated that the decolorization rate of the dye is related to the

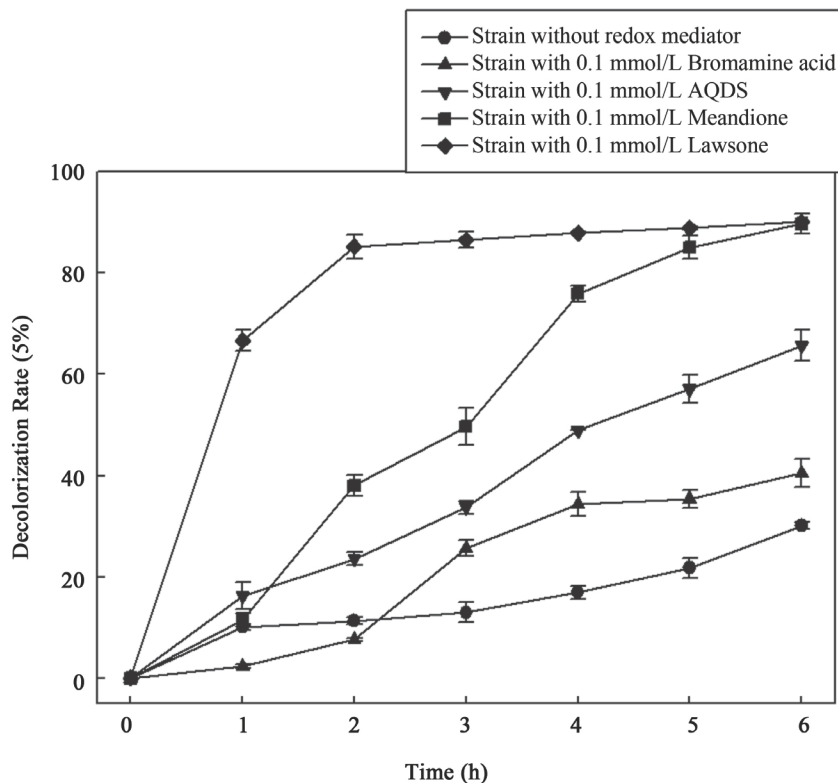


Figure 1. The decolorization rate of Methyl Orange by different mediators.

concentration of the anthraquinone mediator [14]. In this study, the effects of different concentrations of the mediator on the decolorization of azo dyes were determined (Figure 2). As shown in Figure 2, different concentrations of Lawsone added to the system can increase the decolorization rate of the dye. The maximum decolorizing rate was observed when the concentration was 0.1 mmol/L, and about 95% Methyl Orange was degraded within 3 h. (With a specific decolorization rate of 6.87 mg/g cell/h.)

The further increase of the Lawsone concentration over 0.1 mmol/L, the stimulating effect on decolorization decreased correspondingly. When the decolorization system contained 0.25 mmol/L Lawsone, only about 76% dye was degraded within 3 h (with a specific decolorization rate of 5.23 mg/g cell/h). These results indicate that 0.1 mmol/L of mediator was suitable for dye decolorization.

The degradation rate of azo dyes should be increased with the concentration of the mediator raised in the low range. However, when the concentration increases to a certain degree, the decolorization efficiency of azo dyes was no longer increased or even decreased. This might be attributed to the lack of electron donors, which limited the reduction rate of anthraquinone mediator. In addition, high concentrations of anthraquinone compounds may inhibit the growth of cells.

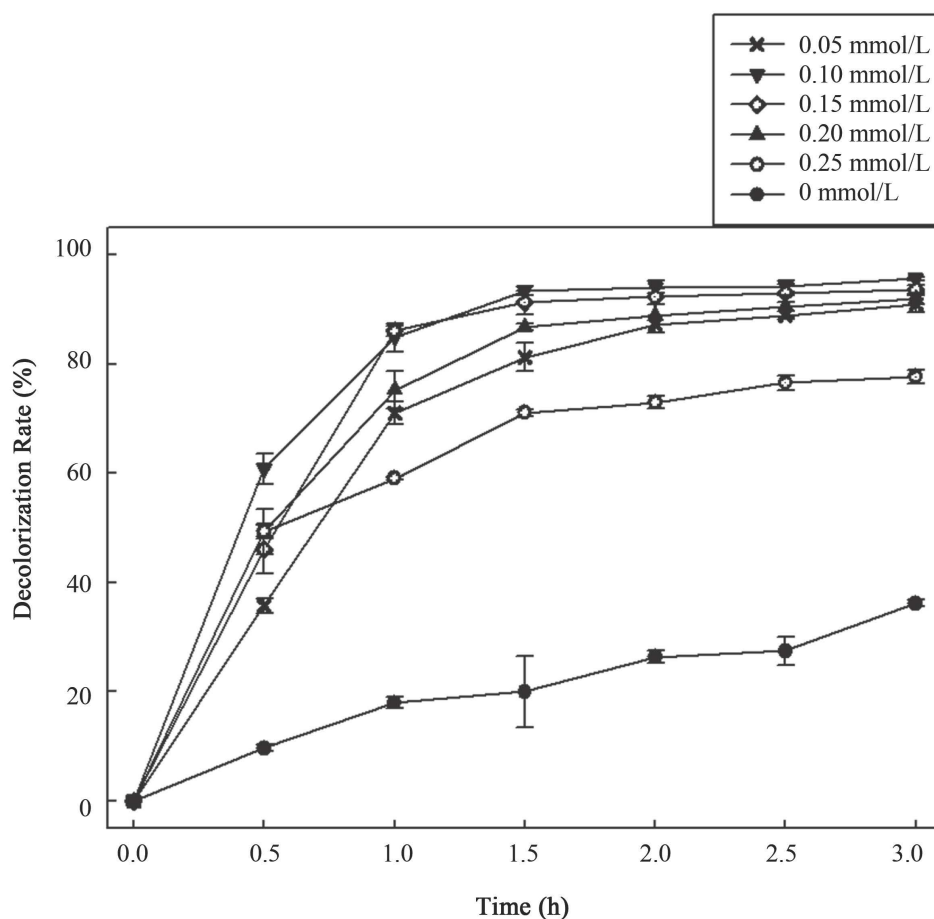


Figure 2. The decolorization rate of the dye by different concentrations of Lawsone.

3.3. The Effect of Physical and Chemical Factors on the Decolorization of Azo Dyes

3.3.1. Effect of pH on Decolorization of Azo Dyes

The decolorizing rate of azo dye in different pH was studied by us, and the result was shown in **Figure 3(a)**. It showed that decolorization of the dye could be observed in a wide range of pH from 4 - 9 by the strain CD-2. The maximum decolorization rate was observed when the culture's pH was 7, and about 92% Methyl Orange was degraded after 3.5 h (with a decolorization rate of 5.24 mg/g cell/h). Both the high pH and low pH influence the decolorization rate of the azo dye. When the culture's pH was 9, only about 37% Methyl Orange was degraded after 3.5 h incubation (with a decolorization rate of 2.13 mg/g cell/h). When the culture's pH was 5, the lowest as 17% Methyl Orange was degraded after 3.5 h (with a decolorization rate of 1.01 mg/g cell/h).

3.3.2. Effect of Temperature on Decolorization of Azo Dyes

In this study, the temperature range of 20°C - 50°C was arranged for the dye decolorizing tests (**Figure 3(b)**). The optimum temperature for the dye decolorization was 37°C, at which over 99% of Methyl Orange was degraded within 2 h (with a decolorization rate of 10.8 mg/g cell/h). The degradation rate decreased greatly when the temperature declined. Only 38% of Methyl orange was decolorized when the temperature dropped to 20°C. However, the decolorized rate remained above 62% at 50°C.

The decolorization rate of azo dyes will be gradually strengthened with the increase of the temperature in a certain range. This is because the higher temperature would provide the higher energy for the dye molecules to break down. However, in the system of biological catalysis decolorization, negative effect will be brought to influence the growth and enzyme activity of the cells at a high temperature. In this study, with the gradual increase of temperature, the degradation rate of azo dyes is reflected in the trend of first increasing and then decreasing. Similar results have been reported, such as Dos Santos *et al.* found that the decolorization rate of azo dye Reactive Red 2 was increased about 2.5 times compared with the 30°C in the anaerobic decolorization system mediated by AQS [15].

3.3.3. Effect of Salinity on Decolorization of Azo Dyes

In this study, the effects of salinity on the degradation rate of azo dye were measured

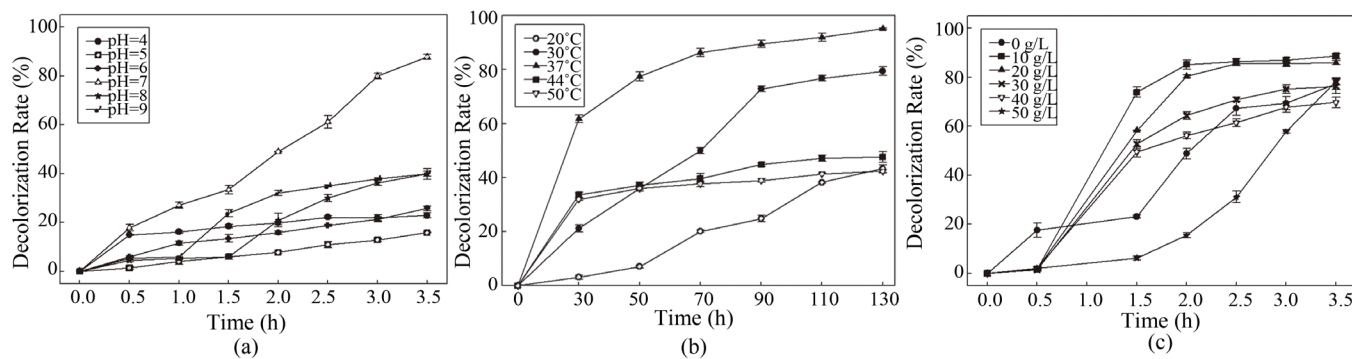


Figure 3. The effects of physical and chemical factors on the decolorization of azo dyes.

(Figure 3(c)). It was observed that salinity is not the main limitation factor for the degradation of Methyl Orange. About 72% of the dye was degraded within 3.5 h in the exist of 10 g/L NaCl, (with a decolorization rate of 4.3 mg/g cell/h). The strain could grow quite well at high salt concentration. Even the concentration of NaCl was 60 g/L, 70% of Methyl Orange could still be degraded within 3.5 h.

3.4. Decolourization of Different Azo Dyes by Strain CD-2

The results suggested that the addition of Lawsone increased the decolorization rates of all tested azo dyes (Table 2). Without a redox mediator, the strain CD-2 could barely decolorize Ponceau S. However, over 82% of the dye was decolorized when the system contained 0.1 mmol/L Lawsone, the specific decolorization rate was 6.59 mg/g cell/h. Similarly, for Gongo Red, when the strain CD-2 was incubated with quinoid compound, the specific decolorization rate increased 2.12 times, from 3.84 to 8.15 mg/g cell/h. When the decolorization system contained Lawsone, the decolorization rate of both Methyl Red and Eriochrome Red B showed 1.30 to 2.06 fold increases, and the specific decolorization rates were 2.30 and 2.97 mg/g cell/h, respectively.

It had been reported that the different efficiencies of various azo dyes decolorization are greatly due to the chemical structures of the azo dyes. The dyes with simple structures and low molecular weights are decolorized faster than those with complex structures. This is likely because the reduction of azo dyes with complex structures was limited by the permeation of the dyes through the cell membrane. When the dye reduction system contained redox mediators, the quinones could transfer reduction equivalents from the periplasmically orientated parts of the respiratory chain to the azo dyes. This mechanism does not require the penetration of the azo dyes through the cell membranes and therefore explains the ability of bacteria to reduce azo dyes with complex structures.

4. Conclusion

In this study, strain CD-2 was used based on its ability to decolorize azo dyes. In addition, quinone compounds were used as redox mediators, and they significantly enhanced the performance of the azo dye decolorization. The results demonstrated that Lawsone was the most effective redox mediator among the quinone compounds tested. The best concentration of Lawsone added in the decolorization system was 0.1 mmol/L. The strain exhibited good decolorization ability at temperatures from 20 °C to 45 °C and

Table 2. The effect of Lawsone on different dye decolorization.

Dye	Reduction rates (mg dye/g cell/h)	
	The strain without Lawsone	The strain with Lawsone
Ponceau S	0.21	6.59
Eriochrome Red B	1.44	2.97
Gongo Red	3.84	8.15
Methyl Red	1.79	2.30

pH values from 4 to 9. Moreover, the strain CD-2 could also degrade Methyl Orange effectively in high salt concentrations. With Lawsone as redox mediator, the strain could decolorize various azo dyes with different chemical structures in a short time. All these results suggested that the strain CD-2 mediated by Lawsone has a great potential for dye-contaminated water treatment.

Acknowledgements

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